PATENT

Attorney Docket No. 40522U

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Sauvaigo, S.
Serial No.: 10/539,769
Filed: December 1, 2005

Confirmation No.: 6482
Art Unit: 1636
Examiner: Joike, Michele K.

For: METHOD FOR THE QUANTITATIVE ASSESSMENT OF GLOBAL AND SPECIFIC DNA REPAIR CAPACITIES OF AT LEAST ONE BIOLOGICAL MEDIUM, AND THE APPLICATIONS THEREOF

DECLARATION UNDER RULE 132

Commissioner of
Patents PO Box 1450
Alexandria, VA 22313-1450

Dear Commissioner:

Now comes Sylvie Sauvaigo, who declares and states that:

1. I have the following degrees and qualifications including the ability to direct PhD theses: PhD in Biology, M.S. in Molecular Chemistry, and a B.S. in Biochemical Engineering.

2. I have been employed by COMMISSARIAT A L'ENERGIE ATOMIQUE (CEA) since October 15th, 1985.

3. I declare that I am experienced in the field of DNA modification, molecular biology and biology, as well as DNA damage and intracellular repair mechanisms as may be seen from the attached truncated curriculum vitae and the publication list attached hereto.

4. I am the named inventor of the above-identified patent application ("the '769 Application").

5. I have reviewed the Office Action dated September 4, 2008, and note that the question of what does and does not constitute supercoiled DNA has been raised by the examiner.

6. The claimed method, and in particular claim 22, uses exclusively supercoiled DNA targets, which contain a random assortment of mutations and lesions which require DNA excision and resynthesis, and, hence, act as a reagent

upon which the DNA excision and resynthesis repair capacities can be both selectively and quantitatively measured.

7. A general methodology for this field is described in the reference book "Molecular Cloning: A Laboratory Manual" by J. Sambrook, E.F. Fritsch and T. Maniatis, Cold Spring Harbor N.Y., (1989). A publication which more precisely illustrates how supercoiled DNA by can be characterized by its electrophoresis pattern on agarose gel is (Quantitation of supercoiled circular content in plasmid DNA in solutions using a fluorescence based method, in Nucleic Acids Research (2000), vol. 28, n° 12, e57).

8. Therefore, such a given circular plasmid will have a specific migration pattern on agarose gel in a given set of conditions. A supercoiled version of this same circular plasmid as in claim 22, will have a different migration pattern in agarose gel due to its supercoiled state.

9. The fact that the target DNA used in the method of claim 22 is supercoiled, is essential as this means that no DNA strand breaks and/or no nuclease digestion has occurred upon the target plasmid. This is because DNA strand breaks inherently lead to a relaxation of super coiled DNA into its base or normal helical structure, as a strand break allows the DNA molecule to attempt to modify its structure so as to remove additional twists.

10. This allows the method according to claim 22, to provide a quantitative assessment of the DNA excision and resynthesis repair mechanisms of the biological sample being tested, while excluding those repair mechanisms which act to repair strand breaks and/or nuclease digestion. Thus, the claimed method is selective for quantitatively assessing DNA excision and resynthesis repair mechanisms.

11. I have also noted that the examiner stated in the Office Action of September 4, 2008, that the DNA used in US 2002/0022228 ('228) is supercoiled, however this is not correct. In '228, experiments are conducted using short synthesized oligonucleotides ("oligos") of between 30-40 bp in length. Such short non-circular oligos inherently are not supercoiled according to the definition of the cited references and as understood by one having ordinary skill in the art. Thus, the topology of these short oligonucleotides cannot be distinguished using electrophoresis pattern migration in agarose gel as described for supercoiled plasmids.

12. Thus, I am of the opinion based on both the literature noted above and the knowledge of those of ordinary skill in the art that the '228 publication does not relate to

supercoiled DNA.

13. I am further of the opinion that one skilled in the art would not have been put in possession of the claimed invention by the '228 publication.

14. The above conclusions are also buttressed by the different methodologies between the claimed invention and the '228 publication. Notably, in the '228 publication short bound single or double stranded oligonucleotides of known sequence are provided which contain one or more defects such as a base mismatch or apurinic site mimicking a mutation which requires excision and resynthesis repair. Samples are then applied to the bound oligos and incorporated labeled nucleotides are used to determine the repair capacity of the tested sample. This methodology only measures DNA repair mechanisms which act upon the selected mutations which diminishes the recorded effects of the other repair mechanisms.

In contrast, in the claimed invention supercoiled plasmids are used to test the repair capacities of a biological sample or medium. These altered plasmids are purified so that only supercoiled plasmids are isolated, which means that plasmids which have undergone a strand break or which more generally have a damaged structure and which probably have been acted upon by a nucleuse cell are eliminated. The remaining supercoiled plasmids which contain a random assortment of other types of other types of mutations and lesions requiring DNA excision and resynthesis can act as a reagent upon which DNA excision and repair capacities of the biological medium can be measured as noted above in a selective and quantitative manner.

15. I am also of the opinion that neither the '228 publication alone or in combination with any one or combination of You et al, Douki, Meselson et al, Chiu et al, Zierdt et al, Gelfrand et al, Yershor et al and/or Randerath et al would have rendered the claimed invention obvious to one skilled in the art at the time the claimed invention was made as none of these cited references, either alone or in combination, would have motivated one skilled in the art to use supercoiled DNA targets exclusively to quantitatively assess the excision and resynthesis capabilities of a biological sample.

16. Finally, I am of the opinion that the claimed invention presents advantages over the method of the '228 publication since although the exact mutations/lesions are unknown for the mutated supercoiled plasmids, because the target plasmids contain a large number of mutation types in a wide variety of locations, such a heterogeneous target reagent can more accurately quantify all of the various DNA excision and resynthesis repair capacities of a

biological medium than can a target reagent consisting of a large number of single type mutations as in '228.

17. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing there from.

Date: February ___, 2009

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Sylvie Sauvaigo

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PRESENT POSITION AND STATUS: CEA Research Director — Senior Researcher

EDUCATION

2007: HEC-Start-Up Institute Diploma (trains entrepreneurs and project managers in the fundamental knowledge of entrepreneurship)

2000: Ability to supervise Research (HDR), University J. Fourier, Grenoble, France 1994: Ph.D in Biology, University J. Fourier, Grenoble, France

1985: Master in Molecular Chemistry, University J. Fourier, Grenoble, France

1984: Engineer Diploma from National Institute of Applied Sciences, Lyon, France (one of the leading French engineer school)

PROFESSIONAL BACKGROUND

2006-present: Leader of the project "Microsystems for measurement of DNA repair" in the CEA program "Technologies for Health", CEA Grenoble, France

1997-present: Senior Researcher in "Nucleic Acids Lesions" Laboratory — Development and use of biochemical methods for measurement of DNA lesions and DNA repair

1985-1997: Project leader at Cis bio international (French diagnostic company) — Development of diagnostic kits for viral infections and cancer

1984: Master degree student, NCI, Ontario, Canada

TEACHING ACTIVITIES

2001-2006: Lectures for Master 2 students (DNA repair)
1986-1992: Setting up of the annual one week seminar "Nucleic Acid Probes" and corresponding courses at French National Institute of Nuclear Sciences and Techniques (INSTN, Paris, France)

SUPERVISION OF STUDENTS

6 Ph.D students
Several Master 1 and Master 2 students

EUROPEAN NETWORKS

1998-2006 ESCODD (European Standards committee on oxidative DNA damage) 2006-2009 COMICS strep (Comet assay and cell array for fast and efficient genotoxicity testing)

PUBLICATION LIST

Millau JF, Raffin AL, Caillat S, Claudet C, Arras G, Ugolin N, Douki T, Ravanat JL, Breton J, Oddos T, Durnontet C, Sarasin A, Chevillard S, Favier A and Sauvaigo S. A microarray to measure repair of damaged plasmids by cell lysates. Lab Chip. 2008, 8, 1713-1722.

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Guerniou V, Rapin D, Millau J.-F., Bufflier E, Favier A, Cadet J and Sauvaigo S. Optimal experimental conditions for the measurement of repair activities of DNA-N-glycosylases contained in cell extracts on a biochip. Applied Nanoscience 2004, 1:39-46

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V. Guerniou, D. Gasparutto, S. Sauvaigo and J. Cadet. New synthesis of 5-carboxy-2'-deoxyuridine and its incorporation into synthetic oligonucleotides. Nucleosides Nucleotides Nucleic Acids. 2003, 22, 1073-1075

A. Collins and ESCODD (2003) Measurement of DNA oxidation in human cells by chromatographic and enzymic methods. Free Rad. Biol. Med., 34, 1089-1099.

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M-J. Richard & S. Sauvaigo. Technique Comete. Imagerie des acides nucleiques et quantification par microscopie photonique (Ronot et Husson Eds.) Tee Lavoisier (2001).

S Sauvaigo, T. Douki, F. Odin, S. Caillat, J.-L. Ravanat and J. Cadet. Analysis of quinolonemediated photosensitization of 2. 2'-deoxyguanosine, calf thymus DNA and cellular DNA: determination of type I, type II and triple-triplet energy transfer mechanism contribution. Photochem. Photobiol., 200, 73, 230-237.

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S. Sauvaigo, R.E. Fretts, R.J. Riopelle and A.E. Lagarde. Autonomous proliferation of MeWo human melanoma cell lines in serum free medium: secretion of growth stimulatoring activities, Int. J. Cancer, 1986, 37, 123-132.

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